# PATENT COOPERATION TREATY

# PCT



## INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

| Applicant's or agent's file reference | FOR FURTHER ACTION | See Form PCT/IPEA/416 |
|---|---|---|
| 042666woJHml | | |
| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
| PCT/EP2004/052789 | 03.11.2004 | 03.11.2003 |

International Patent Classification (IPC) or national classification and IPC
C12N5/06

Applicant
PROBIOGEN AG et al.

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 7 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 3 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

| | | |
|---|---|---|
| ☒ | Box No. I | Basis of the opinion |
| ☐ | Box No. II | Priority |
| ☐ | Box No. III | Non-establishment of opinion with regard to novelty, inventive step and industrial applicability |
| ☐ | Box No. IV | Lack of unity of invention |
| ☒ | Box No. V | Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement |
| ☐ | Box No. VI | Certain documents cited |
| ☐ | Box No. VII | Certain defects in the international application |
| ☒ | Box No. VIII | Certain observations on the international application |

| Date of submission of the demand | Date of completion of this report |
|---|---|
| 25.08.2005 | 23.12.2005 |

Name and mailing address of the international preliminary examining authority:
European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized Officer
Loubradou-Bourges, N
Telephone No. +49 89 2399-7342

# INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/EP2004/052789

## Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

**Description, Pages**

1-37 as originally filed

**Sequence listings part of the description, Pages**

1-16 as originally filed

**Claims, Numbers**

1-13 received on 12.12.2005 with letter of 12.12.2005

**Drawings, Sheets**

1/6-6/6 as originally filed

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

**Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Yes: | Claims | 3-7 yes ; 1-2, 8-13 (see SS, section VIII) |
| | No: | Claims | |
| Inventive step (IS) | Yes: | Claims | 3-7 yes ; 1-2, 8-13 (see SS, section VIII) |
| | No: | Claims | |
| Industrial applicability (IA) | Yes: | Claims | 3-7 yes ; 1-2, 8-13 (see SS, section VIII) |
| | No: | Claims | |

2. Citations and explanations (Rule 70.7):

**see separate sheet**

**Box No. VIII Certain observations on the international application**

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

**see separate sheet**

## Supplemental Box relating to Sequence Listing

**Continuation of Box I, item 2:**

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed

   ☐ filed together with the international application in computer readable form

   ☒ furnished subsequently to this Authority for the purposes of search and/or examination

   ☒ received by this Authority as an amendment on

2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

After a Written Opinion issued by the IPEA, a new set of claims 1-13 was filed.
This set of claims is allowable under Art.34(2)(b) PCT.
The comments of the applicant have been taken into account when drafting the present IPER.

Reference is made to the following documents :

D1: KIM H ET AL: 'ALTERATIONS IN P53 AND E2F-1 FUNCTION COMMON TO IMMORTALIZED CHICKEN EMBRYO FIBROBLASTS' ONCOGENE, BASINGSTOKE, HANTS, GB, vol. 20, no. 21, 2001, pages 2671-2682, XP001157349 ISSN: 0950-9232

D2: BENNETT MARTIN R ET AL: 'Cooperative interactions between RB and p53 regulate cell proliferation, cell senescence, and apoptosis in human vascular smooth muscle cells from atherosclerotic plaques' CIRCULATION RESEARCH, vol. 82, no. 6, 6 April 1998 (1998-04-06), pages 704-712, XP002275529 ISSN: 0009-7330

D3: WAZER DAVID E ET AL: 'Immortalization of distinct human mammary epithelial cell types by human papilloma virus 16 E6 or E7' PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 92, no. 9, 1995, pages 3687-3691, XP002275530 1995 ISSN: 0027-8424

D4: WILLIAMS BART O ET AL: 'Cooperative tumorigenic effects of germline mutations in Rb and p53' NATURE GENETICS, vol. 7, no. 4, 1994, pages 480-484, XP009028188 ISSN: 1061-4036

## Section V

1. The subject-matter of claims 3-7 all, and of part of claims 8-13 referring back thereto meets the requirements of the PCT with respect to novelty and inventive step for the following reasons :

D1 (see abstract) relates to immortalized chicken embryo fibroblast cell lines which have been established in continuous cell culture. The expression pattern of p53 and

E2F-1 has been tested, showing a down and up-regulation, respectively. The E2F-1 factor is known to be involved in the pRb pathway.
The subject-matter of the present application (claims relating to cells and derived subject-matter) differs from the closest prior art D1 in that the immortalized cellular line is **transformed** with genes providing an alteration in the p53 and the pRb pathways.
In D2 and D3 this modification is achieved by retroviral infection and in D4 by breeding.
Thus, D4 does not offer the skilled artisan any possibility for a targeted modification of said metabolic pathways, On the other hand, the transformation methods chosen in D2 and D3 are not qualified for suppression of virus production which is a *conditio sine qua non* for the production of vaccine. They bear the risk of mobilization of the transforming genes. This is due to the fact that in retroviral infection the transforming genes have to be flanked by LTR's. A vaccine potentially contaminated with mobile transforming genes cannot be applied to human recipients. This risk is abrogated by the use of **non-viral transfection** as specified in claims 4-5. Neither D1 nor D2-D4 teaches or suggests a possibility how to immortalize cells with viral genes and at the same tine to inhibit virus production by said cells.
As a conclusion, retroviral transduction is not an option to avoid activation of the transduced factors and thereby increasing the safety profile or the cell. D2 and D3 therefore do not preclude an inventive step.

Additionally, D2 and D3 demonstrate that success with one approach does not imply successful realization of cell immortalization with another approach. The desired activity of the proteins from a human virus as described in claims 4-5 in the avian target cell was unexpected.

The claimed subject-matter therefore encompasses an inventive step.

**Section VIII**

1. A fundamental objection under Art.6 in combination with Art.5 PCT arise with respect to claims 1-2 since the matter for which protection is sought is not clearly defined. The functional statements of affecting the function of the retinoblastoma and the p53

protein (claim 1), overcoming G1 checkpoint control and preventing apoptosis induced by a gene (claim 2), mediating disruption of complexes between retinoblastoma proteins and E2F transcription factors, and preventing transcriptional activation by p53 (claim 1) do not enable the skilled person to determine which technical features are necessary to perform the stated functions.
This objection can in no way be overcome by the passages cited by the applicant, p.9 §2-p.10§4 and p.24§4-p.25§2, since said passages, although describing some examples of genes/proteins fulfilling the above mentioned criteria does not enable the skilled person to determine how to perform the stated function over the whole scope claimed. The definition of the genes disclosed in claims 1-2 is considered to be merely a definition of a result to be achieved and a statement of desiderata.
The same applies correspondingly to parts of claims 8-13 referring back to claims 1-2.

2. Further clarity objections are raised (Art.6 PCT):
The expressions and terms "or the like", "etc." render the scope for which protection is sought unclear.

PCT/EP2004/052789
ProBioGen AG

JH/PCH/cw
December 12, 2005

**Claims**

1. An avian cell line immortalized by non-viral transfection with a combination of viral and/or cellular genes (gene(s)), at least one first gene affecting the function of the retinoblastoma protein by mediating disruption of complexes between retinoblastoma proteins and E2F transcription factors and at least one second gene affecting the p53 protein or a family member thereof, wherein the second gene is a viral gene coding for a protein preventing induction of growth arrest and apoptosis by p53, or is a cellular gene preventing growth arrest and apoptosis by p53.

2. The avian cell line of claim 1, wherein the first gene overcomes G1 checkpoint control and the second gene prevents apoptosis induced by the first gene.

3. The cell line according to claim 1 or 2, wherein
(i) the cell line is derived from embryonic or hatched chicken, duck, goose or quail, preferably from chicken or duck; and/or
(ii) the cells subjected to immortalization are primary cells including fibroblasts, cells from isolated body segments (somites) or separated individual organs including neuronal, brain, retina, kidney, liver, heart, muscle and extraembryonic tissues and membranes protecting the embryo; and/or
(iii) the immortalization by non-viral transfection, includes, but is not limited to, liposome and dendrimer-mediated transfection and electroporation; and/or
(iv) the first gene is a viral gene mediating disruption of complexes between retinoblastoma proteins and E2F transcription factors such as an adenovirus E1A gene from mastadenoviruses, preferably from mastadenoviruses of group C, an E7 gene of papillomaviruses, preferably from low-risk human papilloma virus (HPV) (such as HPV 1, HPV6 and HPV11, but not HPV16 and HPV18), an orf 22 gene of avian adenoviruses, E43 open reading frames from ovine attadenovirus, etc.; or is a cellular gene mediating disruption of complexes between retinoblastoma proteins and E2F transcription factors such as Cyclins D1, D2 or D3, a mutated CDK4 not susceptible to inactivation by p16INK4a, etc.; and/or

(v) the second gene is a viral gene coding for a protein preventing induction of growth arrest and apoptosis by p53 such as the adenovirus E1B55K protein of all groups, GAM-1 of CELO, the E6 protein of papillomaviruses, preferably those of the low-risk HPV (such as HPV 1, HPV6 and HPV11, but not HPV16 and HPV18), or is a cellular gene preventing growth arrest and apoptosis by p53 such as mdm2, etc.; and/or

(vi) the first gene and second gene are separated spatially by heterologous sequences or are located on different nucleic acid segments or plasmids.

4. The cell line of claim 3, which is immortalized with

(i) the E1A (first gene) and E1B (second gene) region of an adenovirus from the genus Mastadenovirus, preferably said E1A and E1B region is derived from adenovirus 5, more preferably said E1A regions have the sequence of bp 1193 to 2309 of SEQ ID NO:7 or the sequence complementary to bp 4230 to 3113 of SEQ ID NO:9, and said E1B regions have the sequence of bp 1145 to 3007 of SEQ ID NO:8 or the sequence complementary to bp 2345 to 550 of SEQ ID NO:9; and/or

(ii) the genes orf22 (first gene) and GAM-1 (second gene) from an adenovirus, preferably from the genus aviadenovirus CELO, which preferably have the sequence represented by the sequence complementary to bp 1252 to 635 of SEQ ID NO:10, and the sequence complementary to bp 3138 to 2290 of SEQ ID NO:10, respectively; and/or

(iii) combinations of nucleic acids encoding E1A and/or E1B with GAM-1 and/or Orf22 as defined in (i) and (ii) above.

5. The cell line according to anyone of claims 1 to 4, which

(i) additionally carries non-natural functional sequences including, but not limited to, transgenes such as genes complementing deficient viruses (e.g. EBNA1, etc.), promoters (e.g. PGK-, EF1.alpha-, CMV-promoter, tk-promoter, etc.), enhancers (e.g. RSV-LTR), selection markers such as neomycin-resistance, puromycin-resistance, etc.; and/or

(ii) is suitable for production of biologicals or viruses including vaccine strains and recombinant viral vectors.

6. The cell line according to anyone of claims 1 to 5, which

(i) is free of reverse transcriptase activity; and/or
(ii) is derived from immortalization of a primary cell originating from duck embryos or hatched ducks; and/or
(iii) is derived from extraembryonic membrane; and/or
(iv) is cultivated in a chemically defined medium which is preferably free of animal serum.

7. The cell line of claims 1 or 6 which is avian cell line 12A07-A10 (DSM ACC2695).

8. A method for preparing a cell line according to anyone of claims 1 to 7, which comprises transforming/transfecting a starting cell with the first and second gene.

9. The method of claim 8 which comprises non-viral transfection of the starting cell.

10. Use of the cell line according to anyone of claims 1 to 7 for the production of biologicals or viruses.

11. A method for producing viruses which comprises
(i) contacting said viruses with a cell line according to any one of claims 1 to 7 and/or
(ii) cultivating said viruses on said cell line.

12. The method of claim 11 for producing a pox virus, preferably strain MVA, in a duck cell line, preferably a cell line originating from duck somites or duck neuronal tissue, even more preferred from duck retina.

13. A method for producing recombinant proteins which comprises
(i) introducing a gene coding for a recombinant protein, operably linked to a promoter, into a cell line according to any of claims 1 to 7,
(ii) cultivating said modified cell line and
(iii) harvesting the recombinant protein.